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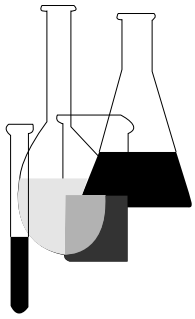
Product Properties Test Guidelines

OPPTS 830.7560 Partition Coefficient (*n*-Octanol/Water), Generator Column Method



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 830.7560 Partition coefficient (*n*-Octanol/water), generator column method.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are the OPPT guideline under 40 CFR 796.1550 Octanol/water partition coefficient generator column method, and OPP guideline 63–11 Octanol/water partition coefficient (Pesticide Assessment Guidelines, Subdivision D: Product Chemistry, EPA Report 540/9–82–018, October 1982).

(b) **Introduction**—(1) **Purpose.** (i) Since the pioneering work of Fujita and Hansch (see paragraph (e)(7) of this guideline) in the measurement and estimation of the octanol/water partition coefficient (K_{ow}), this property has become the cornerstone of a myriad of structure-activity relationships (SAR). Hansch and Leo (see paragraph (e)(8) of this guideline) have used the coefficient extensively for correlating structural changes in drugs with changes observed in biological, biochemical, or toxic effects. These correlations are then used to predict the effect of a new drug for which a K_{ow} could be measured.

(ii) In the study of the environmental fate of organic chemicals, the octanol/water partition coefficient has become a key parameter. It has been shown to be correlated to water solubility, soil/sediment sorption coefficient, and bioconcentration. The importance of this property to SAR is indicated by its discussion in the first chapter of Lyman, Reehl and Rosenblatt's (see paragraph (e)(11) of this guideline). These authors consider the measurement or estimation of the octanol/water partition coefficient to be the necessary first step in assessing the fate of chemicals.

(iii) Of the three properties that can be estimated from K_{ow}, water solubility is the most important because it affects both the fate and transport of chemicals. For example, highly soluble chemicals become quickly distributed by the hydrologic cycle, have low sorption coefficients for soils and sediments, and tend to be more easily degraded by microorganisms. In addition, chemical transformation processes such as hydrolysis, direct photolysis, and indirect photolysis (oxidation) tend to occur more readily if a compound is soluble.

(iv) Direct correlations between K_{ow} and both the soil/sediment sorption coefficient and the bioconcentration factor are to be expected. In these cases compounds that are more soluble in octanol (more hydrophobic and lipophilic) would be expected to partition out of the water and into the organic portion of soils/sediments and into lipophilic tissue. The relationship between K_{ow} and the bioconcentration factor, as developed by Neely

et al. (see paragraph (e)(15) of this guideline), and other similar relationships, are the principal means of estimating bioconcentration factors. These factors are then used to predict the potential for a chemical to accumulate in living tissue.

(v) This test guideline describes a method for determining the octanol/water partition coefficient based on the dynamic coupled column liquid chromatographic technique, a technique commonly referred to as the generator column method. This method was the basis for a previous test guideline for water solubility, OPPTS 830.7860 and closely follows that section. The method described herein can be used in place of the standard shake-flask method described in OPPTS 830.7550 for compounds with a $\log_{10}K_{ow}$ greater than 1.0.

(2) **Definitions and units.** (i) The *octanol/water partition coefficient* (K_{ow}) is defined as the ratio of the molar concentrations of a chemical in n-octanol and water, in dilute solution. The coefficient K_{ow} is a constant for a given chemical at a given temperature. Since K_{ow} is the ratio of two molar concentrations, it is a dimensionless quantity. Sometimes K_{ow} is reported as the decadic logarithm ($\log_{10}K_{ow}$). The mathematical statement of K_{ow} is:

$$K_{ow} = C_{octanol}/C_{water}$$

where $C_{octanol}$ and C_{water} are the molar concentration of the solute in *n*-octanol and water, respectively, at a given temperature. This test procedure determines K_{ow} at 25±0.05 °C.

(ii) A *generator column* is used to partition the test substance between the octanol and water phases. The column in figure 1 under paragraph (c)(1)(i)(A)(*2*) of this guideline is packed with a solid support and is coated with the test substance at a fixed concentration in n-octanol. The test substance is eluted from the column with water and the aqueous solution leaving the column represents the equilibrium concentration of the test substance that has partitioned from the octanol phase into the water phase. Preparation of the generator column is described under paragraph (c)(1)(i) of this guideline.

(iii) An *extractor column* is used to extract the solute from the aqueous solution produced by the generator column. After extraction onto a bonded chromatographic support, the solute is eluted with a solvent/water mixture and subsequently analyzed by high-performance liquid chromatography (HPLC), gas chromatography (GC), or any other analytical procedure. A detailed description of the preparation of the extractor column is given in paragraph (c)(1)(i) of this guideline.

(iv) The *sample loop* is a 1⁄16 in. O.D. (1.6 mm) stainless steel tube with an internal volume between 20 and 50 µL. The loop is attached to the sample injection valve of the HPLC and is used to inject standard

solutions into the mobile phase of the HPLC when determining the response factor for the recording integrator. The exact volume of the loop must be determined as described in paragraph (c)(3)(iii)(C)(*1*) of this guideline when the HPLC method is used.

(v) The *response factor* (RF) is the solute concentration required to give a one unit area chromatographic peak or one unit output from the HPLC recording integrator at a particular recorder and detector attenuation. The factor is required to convert from units of area to units of concentration. The determination of the response factor is given in paragraph (c)(3)(iii)(C)(*2*) of this guideline.

(3) **Principle of the test method.** (i) This test method is based on the dynamic coupled column liquid chromatographic (DCCLC) technique for determining the aqueous solubility of organic compounds that was initially developed by May et al. (see paragraphs (e)(12) and (e)(13) of this guideline), modified by DeVoe et al. (see paragraph (e)(6) of this guideline), and finalized by Wasik et al. (see paragraph (e)(20) of this guideline). The DCCLC technique utilizes a generator column, extractor column and HPLC coupled or interconnected to provide a continuous closed flow system. Aqueous solutions of the test compound are produced by pumping water through the generator column that is packed with a solid support coated with an approximately 1.0 percent (w/w) solution of the compound in octanol. The aqueous solution leaving the column represents the equilibrium concentration of the test chemical which has partitioned from the octanol phase into the water phase. The compound is extracted from the aqueous solution onto an extractor column, then eluted from the extractor column with a solvent/water mixture and subsequently analyzed by HPLC using a variable wavelength UV absorption detector operating at a suitable wavelength. Chromatogram peaks are recorded and integrated using a recording integrator. The concentration of the compound in the effluent from the generator column is determined from the mass of the compound (solute) extracted from a measured volume of water (solvent). The octanol/water partition coefficient is calculated from the ratio of the molar concentration of the solute in the 1.0 percent (w/w) octanol and molar concentration of the solute in water as determined using the generator column technique.

(ii) Since the HPLC method is only applicable to compounds that absorb in the UV, an alternate GC method, or any other reliable quantitative procedure (which must be approved by the OPPTS), is used for those compounds that do not absorb in the UV. In the GC method the saturated solutions produced in the generator column are extracted using an appropriate organic solvent that is subsequently injected into the GC, or any other suitable analytical device,for analysis of the test compound.

(4) **Reference chemicals.** (i) Columns 2, 3, 4, and 5 of table 1 in paragraph (b)(4)(ii) of this guideline list the experimental values of the

decadic logarithm of the octanol/water partition coefficient ($\log_{10}K_{ow}$) at 25 °C for a number of organic chemicals as obtained from the scientific literature. These values were obtained by one of the following experimental methods: Shake-flask; generator column; reverse-phase high-performance liquid chromatography (HPLC); or reverse-phase thin-layer chromatography, as indicated in the footnotes following each literature citation. The fragment constant method of Hansch and Leo, under paragraph (e)(8) of this guideline, has been computerized; the PC version is called CLOGP3, cited under paragraph (e)(9) of this guideline, and was used to estimate $\log_{10}K_{ow}$ for a number of the chemicals. These values are listed in column 6 of table 1 in paragraph (b)(4)(ii) of this guideline. The estimation method of Hawker and Connell under paragraph (e)(10) of this guideline, correlates $\log_{10}K_{ow}$ with the total surface area of the molecule and was used to estimate $\log_{10}K_{ow}$ for biphenyl and the chlorinated biphenyls. These estimated values are listed in column 7 of table 1 in paragraph (b)(4)(ii) of this guideline. Recommended values of $\log_{10}K_{ow}$ were obtained by critically analyzing the available experimental and estimated values and averaging the best data. These recommended values are listed in column 8 of table 1 in paragraph (b)(4)(ii) of this guideline.

(ii) The recommended values listed in the following table 1 have been provided primarily so that the generator column method can be calibrated and to allow the chemical laboratory the opportunity to compare its results with these values. The testing laboratory has the option of choosing its reference chemicals, but references must be given to establish the validity of the measured values of $\log_{10}K_{ow}$.

Table 1.—Octanol/Water Partition Coefficient at 25 C for Some Reference Compounds

Chemical	Experimental $\log_{10}K_{ow}$				Estimated $\log_{10}K_{ow}$		Recommended $\log_{10}K_{ow}$
	Hansch and Leo[1]	Generator Column Method	Banerjee[2]	Other values	Hansch and Leo[3]	Hawker and Connell[4]	
Ethyl acetate	0.73, 0.66	0.68[5]	—	—	0.671	—	0.685[17]
1-Butanol	0.88, 0.89, 0.32, 0.88	0.785[5]	—	—	0.823	—	0.852[23]
1-Pentanol	1.28, 1.40	1,53[5]	—	—	1.35	—	1.39[17]
Nitrobenzene	1.85, 1.88, 1.79	1.85[5]	1.83	1.82[6]	1.89	—	1.84[17]
Benzene	2.15, 2.13	—	2.12	—	2.14	—	2.14[17]
Trichloroethylene	2.29	2.53[5]	2.42	—	2.27	—	2.38[17]
Chlorobenzene	2.84, 2.46	2.98[7]	—	2.84[8]	2.86	—	2.80[18]
o-Dichlorobenzene	3.38	3.38[7]	3.40	3.38[8]	3.57	—	3.42[17]
n-Propylbenzene	3.66, 3.66, 3.68, 3.57	3.69[5]	—	—	3.85	—	3.69[17]
Biphenyl	3.95, 4.17, 4.09, 4.04	3.67[7], 3.89[9], 3.79[10]	4.04	3.75[6]	4.03	4.09	3.96[17]
2-Chlorobiphenyl	—	4.50[7], 4.38[9]	—	3.90[10], 3.75[11], 4.59[12], 4.54[13]	—	4.99	4.49[19]
1,2,3,5-Tetrachlorobenzene	—	4.65[7]	4.46	—	4.99	—	4.70[17]

Table 1.—Octanol/Water Partition Coefficient at 25 C for Some Reference Compounds—Continued

Chemical	Experimental $\log_{10}K_{ow}$				Estimated $\log_{10}K_{ow}$		Recommended $\log_{10}K_{ow}$
	Hansch and Leo[1]	Generator Column Method	Banerjee[2]	Other values	Hansch and Leo[3]	Hawker and Connell[4]	
2,2′-Dichlorobiphenyl	—	4.90[9]	—	4.90[9], 3.63[10], 3.55[11], 4.51[14], 5.02[15]	—	4.65	4.80[20]
Pentachlorobenzene	—	5.03[7]	4.94	—	5.71	—	4.99[24]
2,4,5-Trichlorobiphenyl	—	5.51[7], 5.81[9]	—	5.67[10], 5.86[10], 5.77[15]	—	5.60	5.70[17]
2,3,4,5-Tetrachlorobiphenyl	—	6.18[4], 5.72[7]	—	—	—	6.04	5.98[17]
2,2′,4,5,5′-Pentachlorobi-phenyl	6.11	6.50[9], 5.92[7]	—	6.11[13], 6.85[12]	—	6.38	6.31[17]
2,2′,3,3′,6,6′-Hexachloro-biphenyl	—	5.76[4], 6.63[7], 6.81[9]	—	—	—	6.22	6.36[17]
2,2′,3,3′,4,4′,6-Heptachlorobiphenyl	—	6.68[7]	—	—	—	7.11	6.90[17]
2,2′,3,3′,5,5′,6,6′-Octachlorobiphenyl	—	7.11[7], 7.14[9]	—	8.42[12]	—	7.24	7.16[21]
2,2′,3,3′,4, 4′,5,6,6′-Nona-chlorobiphenyl	—	7.52[4]	—	—	—	7.74	7.63[17]
2,2′,3,3′,4, 5,5′6,6′-Nona-chlorobiphenyl	—	8.16[7]	—	—	—	7.71	7.94[17]
Decachlorobiphenyl	—	8.26[7], 8.20[9]	—	9.60[12]	—	8.18	8.21[22]

[1] Hansch and Leo (1979). Shake-flask method under paragraph (e)(8) of this guideline.

[2] Banerjee, Yalkowski, and Valvani (1980). Shake-flask method under paragraph (e)(1) of this guideline.

[3] Hansch and Leo (1984). Estimates $\log_{10}K_{ow}$ using the CLogP3 computer program under paragraph (e)(9) of this guideline.

[4] Hawker and Connell (1988). Generator column method and an estimation method correlating $\log_{10}K_{ow}$ with the total surface area of the molecule under paragraph (e)(10) of this guideline.

[5] Tewari et al. (1982). Generator column method under paragraph (e)(17) of this guideline.

[6] Veith, Austin, and Morris (1979). Reverse-phase HPLC method under paragraph (e)(19) of this guideline.

[7] Miller et al. (1984). Generator column method under paragraph (e)(14) of this guideline.

[8] Chiou and Schmedding (1982). Shake-flask method under paragraph (e)(4) of this guideline.

[9] Woodburn, Doucette, and Andren (1984). Generator column method under paragraph (e)(22) of this guideline.

[10] Rapaport and Eisenreich (1984). Reverse-phase HPLC method under paragraph (e)(16) of this guideline.

[11] Woodburn (1982). Reverse-phase HPLC method under paragraph (e)(21) of this guideline.

[12] Bruggemann, Van der Steen, and Hutzinger (1978). Shake-flask method under paragraph (e)(2) of this guideline.

[13] Tulp and Hutzinger (1982). Shake-flask method under paragraph (e)(18) of this guideline.

[14] Chiou, Porter, and Schmedding (1983). Shake-flask method under paragraph (e)(5) of this guideline.

[15] Bruggemann, Van Der Steen , and Hutzinger (1982). Reverse-phase thin-layer chromatography under paragraph (e)(2) of this guideline.

[16] Chiou et al. (1977). Shake-flask method under paragraph (e)(3) of this guideline.

[17] Average value using all the data.

[18] Average value using all the data except the datum point 2.46.

[19] Average value using all the data except the data points 3.90 and 3.75.

[20] Average value using all the data except the data points 3.63 and 3.55.

[21] Average value using all the data except the datum point 8.42.

[22] Average value using all the data except the datum point 9.60.

[23] Average value using all the data except the datum point 0.32.

[24] Average value using all the data excluding the estimated datum point 5.71.

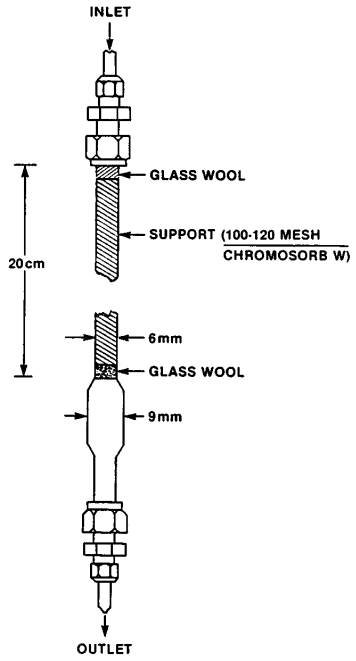
(5) **Applicability and specificity.** The test guideline is designed to determine the octanol/water partition coefficient of solid or liquid organic chemicals in the range $\log_{10}K_{ow}$ 1.0 to >6.0 (10 to >10^6).

(b) **Test procedure**—(1) **Test conditions**—(i) **Special laboratory equipment.** (A)(*1*) Generator column. Either of two different methods for connecting to the generator column shall be used depending on whether

the eluted aqueous phase is analyzed by HPLC (Procedure A, as described in paragraph (b)(3)(iii) of this guideline) or by solvent extraction followed by GC analysis, or any other reliable method, of solvent extract (Procedure B, as described in paragraph (b)(3)(iv) of this guideline).

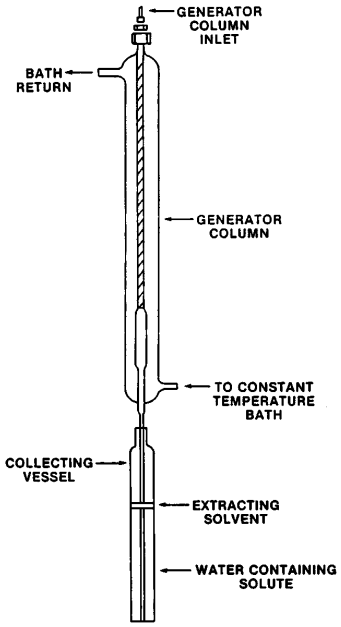
(*2*) The design of the generator column is shown in the following figure 1:

FIGURE 1—GENERATOR COLUMN



The column consists of a 6 mm (¼-in) O.D. Pyrex tube joined to a short enlarged section of 9 mm Pyrex tubing which in turn is connected to another section of 6 mm (¼-in) O.D. Pyrex tubing. Connections to the inlet Teflon tubing (⅛-in O.D.) and to the outlet stainless steel tubing (1⁄16-in O.D.) are made by means of stainless steel fittings with Teflon ferrules. The column is enclosed in a water jacket for temperature control as shown in the following figure 2:

FIGURE 2—SETUP SHOWING GENERATOR COLUMN ENCLOSED IN A WATER JACKET AND OVERALL ARRANGEMENT OF THE APPARATUS USED IN GC METHOD



(B) Constant temperature bath with circulation pump-bath and capable of controlling temperature to 25±0.05 °C. (Procedures A and B, as described in paragraphs (b)(3)(iii) and (b)(3)(iv) of this guideline).

(C) High pressure liquid chromatograph equipped with a variable wavelength UV absorption detector operating at a suitable wavelength and a recording integrator (Procedure A, as described in paragraph (b)(3)(iii) of this guideline).

(D) Extractor column—6.6 × 0.6 cm stainless steel tube with end fittings containing 5 micron frits filled with a superficially porous phase packing (Bondapack C_{18} Corasil: Water Associates) (Procedure A, as described in paragraph (b)(3)(iii) of this guideline).

(E) Two 6-port high pressure rotary switching valves (Procedure A, as described in paragraph (b)(3)(iii) of this guideline).

(F) Collection vessel: An 8 × ¾ in section of Pyrex tubing with a flat bottom connected to a short section of ⅜-in O.D. borosilicate glass tubing. The collecting vessel is sealed with a ⅜-in Teflon cap fitting (Procedure B, as described in paragraph (b)(3)(iv) of this guideline).

(G) GC, or any other reliable analytic equipment, equipped with a detector sensitive to the solute of interest (Procedure B, as described in paragraph (b)(3)(iv) of this guideline).

(ii) **Purity of octanol and water.** Purified n-octanol, described in paragraph (c)(2)(i) of this guideline, and water meeting ASTM Type II standards, or an equivalent grade, are recommended to minimize the effects of dissolved salts and other impurities. ASTM Type II water is described in ASTM D 1193–77 (Reapproved 1983), ''Standard Specification for Reagent Water''. Copies may be obtained from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(iii) **Purity of solvents.** It is important that all solvents used in this method be reagent or HPLC grade and contain no impurities which could interfere with the determination of the test compound.

(iv) **Reference compounds.** In order to ensure that the HPLC system is working properly, at least two of the reference compounds listed in table 1 in paragraph (b)(4)(ii) of this guideline should be run. Reference compounds shall be reagent or HPLC grade to avoid interference by impurities.

(2) **Preparation of reagents and solutions**—(i) **Octanol and water.** Very pure octanol can be obtained as follows: Wash pure octanol (minimum 98 percent pure) sequentially with 0.1N H_2SO_4, with 0.1N NaOH, then with distilled water until neutral. Dry the octanol with magnesium sulfate and distill twice in a good distillation column under reduced pressure [b.p. about 80 °C at 0.27 kPa (2 torr)]. The octanol produced should be at least 99.9 percent pure. Alternatively, a grade equivalent to Fisher Scientific Co. No. A–402 ''Certified Octanol–1'' can be used. Reagent grade water shall be used throughout the test procedure; that is, ASTM Type II water as described in paragraph (b)(1)(ii) of this guideline.

(ii) **Presaturated water.** Prepare presaturated water with octanol to minimize the depletion of octanol from the column when measuring the octanol/water partition coefficient of a test chemical. This is very important when the test chemical is lipophilic and the $\log_{10}K_{ow}$ >4.

(3) **Performance of the test.** Initially, an approximately 1.0 percent (w/w) solution of the test substance in octanol is prepared. Precise measurement of the solute concentration in this solution is required for the K_{ow} calculation. Subsequently, the 1.0 percent (w/w) solution is coated on the generator column and using either Procedure A or Procedure B as de-

scribed in paragraphs (b)(3)(iii) and (b)(3)(iv) of this guideline, the molar concentration of the test substance in reagent grade water is determined.

(i) **Test solution.** The test solution consists of an approximately 1.0 percent (w/w) solution of the test substance in octanol. A sufficient quantity (about 10–20 mL) of the test solution should be prepared to coat the generator column. The solution is prepared by accurately weighing out, using a tared bottle, quantities of both the test substance and octanol required to make a 1.0 percent (w/w) solution. When the weights are measured precisely (to the nearest 0.1 mg), knowing the density of octanol (0.827 g/mL at 25 °C), then the molar concentration of the test substance in the octanol is sufficiently accurate for the purposes of the test procedure. If desired, however, a separate analytical determination (e.g., by GC, or any other reliable analytical method) may be used to check the concentration in the test solution. If storage is required, the test solution should be kept stoppered to prevent volatilization of the test chemical.

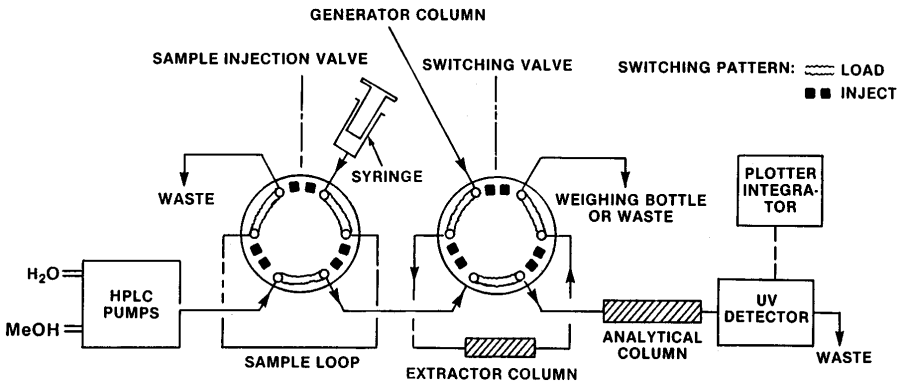
(ii) **Test procedures.** Prior to the determination of the octanol/water partition coefficient of the test chemical, two procedures shall be followed:

(A) The saturated aqueous solution leaving the generator column shall be tested for the presence of an emulsion, using a Tyndall procedure (i.e. light scattering). If colloids are present, they must be removed prior to injection into the extractor column by lowering the flow rate of water.

(B) The efficiency of removal of the solute (the test chemical) by solvent extraction from the extractor column shall be determined and used in the determination of the octanol/water partition coefficient of the test chemical.

(iii) **Procedure A—HPLC method.** (A) Procedure A covers the determination of the aqueous solubility of compounds which absorb in the UV. The HPLC analytical system is shown schematically in the following figure 3:

FIGURE 3—SCHEMATIC OF HPLC—GENERATOR COLUMN FLOW SYSTEM



Two reciprocating piston pumps deliver the mobile phase (water or solvent/water mixture) through two 6-port high pressure rotary valves and a 30 x 0.6 cm C_{18} analytical column to a UV absorption detector operating at a suitable wavelength. Chromatogram peaks are recorded and integrated with a recording integrator. One of the 6-port valves is the sample injection valve used for injecting samples of standard solutions of the solute in an appropriate concentration for determining response factors or standard solutions of basic chromate for determining the sample loop volume. The other 6-port valve in the system serves as a switching valve for the extractor column which is used to remove solute from the aqueous solutions.

(B) The general procedure for analyzing the aqueous phase after equilibration is as follows; a detailed procedure is given in paragraph (c)(3)(iii)(C)(*4*) of this guideline:

(*1*) Direct the aqueous solution from the generator column to ''Waste'' in figure 3 under paragraph (c)(3)(iii)(A) of this guideline with the switching valve in the inject position in order to equilibrate internal surfaces with the solution, thus insuring that the analyzed sample would not be depleted by solute adsorption on surfaces upstream from the valve.

(*2*) At the same time, water is pumped from the HPLC pumps in order to displace the solvent from the extractor column.

(*3*) The switching valve is next changed to the load position to divert a sample of the solution from the generator column through the extractor column, and the liquid leaving the extractor column is collected in a tared weighing bottle. During this extraction step, the HPLC mobile phase is changed to a solvent/water mixture to condition the analytical column.

(*4*) After the desired volume of sample is extracted, the switching valve is returned to the inject position for elution from the extractor column and analysis. Assuming that all of the solute was adsorbed by the extractor column during the extraction step, the chromatographic peak represents all of the solute in the extracted sample, provided that the extraction efficiency is 100 percent. If the extraction efficiency is less than 100 percent, then the extraction efficiency shall be measured and used to determine the actual amount of the solute extracted.

(*5*) The solute concentration in the aqueous phase is calculated from the peak area, the weight of the extracted liquid collected in the weighing bottle, the extraction efficiency, and the response factor.

(C)(*1*) **Determination of the sample loop volume.** Accurate measurement of the sample loop may be accomplished by using the spectrophotometric method of Devoe et al. (1981) under paragraph (e)(6) of this guideline. For this method measure absorbance, $A_{loop,}$ at 373 nm for at least three solutions, each of which is prepared by collecting from the sample valve an appropriate number, n, of loopfuls of an aqueous stock

solution of K_2CrO_4 (1.3 percent by weight) and diluting to 50 mL with 0.2 percent KOH. (For a 20 μL loop, use n = 5; for a 50 μL loop, use n = 2.) Also measure the absorbance, A_{stock}, of the same stock solution after diluting 1:500 with 0.2 percent KOH. Calculate the loop volume to the nearest 0.1 μL using the relation:

$$V_{loop} = (A_{loop}/A_{stock})(10^{-4}/n)$$

(*2*) **Determination of the response factor (RF). (***i*) For all determinations adjust the mobile phase solvent/water ratio and flow rate to obtain a reasonable retention time on the HPLC column. For example, typical concentrations of organic solvent in the mobile phase range from 50 to 100 percent while flow rates range from 1 to 3 mL/min; these conditions often give a 3 to 5 min retention time.

(*ii*) Prepare standard solutions of known concentrations of the solute in a suitable solvent. Concentrations must give a recorder response within the maximum response of the detector. Inject samples of each standard solution into the HPLC system using the calibrated sample loop. Obtain an average peak area from at least three injections of each standard sample at a set detector absorbance unit full scale (AUFS), i.e., at the same absorbance scale attenuation setting.

(*iii*) Calculate the response factor from the following equation:

$$\text{Response Factor (RF)} = \frac{\text{Concentration (M)}}{\text{(Average Area) (AUFS)}}$$

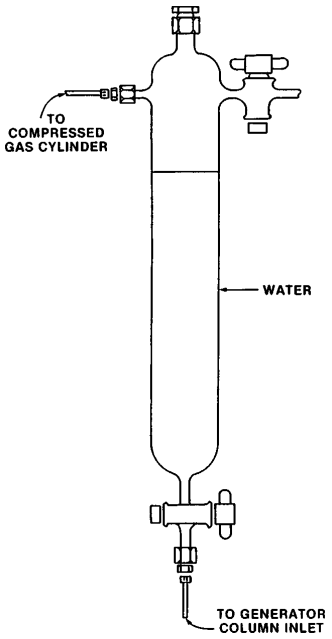
(*3*) **Loading of the generator column.** (*i*) The design of the generator column was described in paragraph (c)(1)(i) of this guideline and is shown in figure 1 under paragraph (c)(1)(i)(A)(*2*) of this guideline. To pack the column, a plug of silanized glass wool is inserted into one end of the 6 mm Pyrex tubing. Silanized diatomaceous silica support (about 0.5g of 100–120 mesh Chromosorb W chromatographic support material) is poured into the tube with tapping and retained with a second plug of silanized glass wool.

(*ii*) The column is loaded by pulling the test solution through the dry support with gentle suction and then allowing the excess solution to drain out. After loading the column, draw water up through the column to remove any entrapped air.

(*4*) **Analysis of the solute.** Use the following procedure to collect and analyze the solute:

(*i*) Pump water to the generator column by means of a minipump or pressurized water reservoir as shown in the following figure 4:

FIGURE 4—WATER RESERVOIR FOR GC METHOD



With the switching valve in figure 3 under paragraph (c)(3)(iii)(A) of this guideline in the inject position (i.e., water to waste), pump water through the generator column at a flow rate of approximately 1 mL/min for approximately 15 min to bring the system into equilibrium.

(*ii*) Flush out the organic solvent that remains in the system from previous runs by changing the mobile phase to 100 percent H_2O and allowing the water to reach the HPLC detector, as indicated by a negative reading. As soon as this occurs, place a 25 mL weighing bottle (weighed to the nearest mg) at the waste position and immediately turn the switching valve to the load position.

(*iii*) Collect an amount of water from the generator column (as determined by trial and error) in the weighing bottle, corresponding to the amount of solute adsorbed by the extractor column that gives a reasonable detector response. During this extraction step, switch back to the original HPLC mobile phase composition, i.e., solvent/water mixture, to condition the HPLC analytical column.

(*iv*) After the desired volume of sample has been extracted, turn the switching valve back to the inject position in figure 3 under paragraph (c)(3)(iii)(A) of this guideline. As soon as the switching valve is turned to the inject position, remove the weighing bottle, cap it and replace it with the waste container; at the same time turn on the recording integrator. The solvent/water mobile phase will elute the solute from the extractor column and transfer the solute to the HPLC analytical column.

(*v*) Determine the weight of water collected to the nearest mg and record the corresponding peak area. Using the same AUFS setting repeat the analysis of the solute at least two more times and determine the average ratio of peak area to grams of water collected. Calculate the solute solubility in water using the following equation:

$$S = (997\ g/L)(RF)(V_{loop})(AUFS)(R)$$

where

S = solubility (M)
RF = response factor
V_{loop} = sample loop volume (L)
R = ratio of area to grams of water.

(iv) **Procedure B—GC Method.** In the GC method, or any other reliable quantitative method, aqueous solutions from the generator column enter a collecting vessel in figure 2 under paragraph (c)(1)(i)(A)(*2*) of this guideline containing a known weight of extracting solvent which is immiscible in water. The outlet of the generator column is positioned such that the aqueous phase always enters below the extracting solvent. After the aqueous phase is collected, the collecting vessel is stoppered and the quantity of aqueous phase is determined by weighing. The solvent and the aqueous phase are equilibrated by slowly rotating the collecting vessel. A small amount of the extracting solvent is then removed and injected into a GC equipped with an appropriate detector. The solute concentration in the aqueous phase is determined from a calibration curve constructed using known concentrations of the solute. The extraction efficiency of the solvent shall be determined in a separate set of experiments.

(A) **Determination of calibration curve.** (*1*) Prepare solute standard solutions of concentrations covering the expected range of the solute solubility. Select a column and optimum GC operating conditions for resolution between the solute and solvent and the solute and extracting solvent. Inject a known volume of each standard solution into the injection port of the GC. For each standard solution determine the average of the ratio R of peak area to volume (in μL) for the chromatographic peak of interest from at least three separate injections.

(*2*) After running all the standard solutions, determine the coefficients, a and b, using linear regression analysis on the equation of concentration (C) vs. R in the form

$$C = aR + b$$

(B) **Loading of the generator column.** The generator column is packed and loaded with solute in the same manner as for the HPLC method in paragraph (c)(3)(iii) of this guideline. As shown in figure 2 under paragraph (c)(1)(i)(A)(*2*) of this guideline, attach approximately 20 cm of straight stainless steel tubing to the bottom of the generator column. Connect the top of the generator column to a water reservoir in figure 4 under paragraph (c)(3)(iii)(C)(*4*)(*i*) of this guideline using Teflon tubing. Use air or nitrogen pressure (5 PSI) from an air or nitrogen cylinder to force water from the reservoir through the column. Collect water in an Erlenmeyer flask for approximately 15 minutes while the solute concentration in water equilibrates; longer time may be required for less soluble compounds.

(C) **Collection and extraction of the solute.** During the equilibration time, add a known weight of extracting solvent to a collection vessel which can be capped. The extracting solvent should cover the bottom of the collection vessel to a depth sufficient to submerge the collecting tube but still maintain 100:1 water/solvent ratio. Record the weight (to the nearest mg) of a collection vessel with cap and extracting solvent. Place the collection vessel under the generator column so that water from the collecting tube enters below the level of the extracting solvent in figure 2 under paragraph (b)(1)(i)(A)(*2*) of this guideline. When the collection vessel is filled, remove it from under the generator column, replace cap, and weigh the filled vessel. Determine the weight of water collected. Before analyzing for the solute, gently rotate the collection vessel contents for approximately 30 min., controlling the rate of rotation so as not to form an emulsion; rotating the flask end over end five times per minute is sufficient. The extraction efficiency of the solvent shall be determined in a separate set of experiments.

(D) **Analysis of the solute.** (*1*) After rotating, allow the collection vessel to stand for approximately 30 minutes; then remove a known volume of the extracting solvent from the vessel using a microliter syringe and inject it into the GC. Record the ratio of peak area to volume injected and, from the regression equation of the calibration line, determine the concentration of solute in the extracting solvent. If the extraction efficiency is not 100 percent, the measured extraction efficiency shall be used to obtain the correct concentration of solute extracted. The molar concentration of solute in water C(M) is determined from the following equation

$$C(M) = (C_{es})\,[d_{H2O}/d_{es}][g_{es}/g_{H2O}]$$

where C_{es} is the molar concentration of solute in extracting solvent, d_{H2O} and d_{es} are the densities in grams per milliliter of water and extracting solvent, respectively, and g_{es} and g_{H2O} are the grams of extracting solvent and water, respectively, contained in the collection vessels.

(*2*) Make replicate injections from each collecting vessel to determine the average solute concentration in water for each vessel. To make sure the generator column has reached equilibrium, run at least two additional (for a total of three) collection vessels and analyze the extracted solute as described above. Calculate C(M) from the average solute concentration in the three vessels.

(*3*) If another analytical method is used in place of the GC, then Procedure B (as described in paragraph (b)(3)(iv) of this guideline) shall be modified and the new analytical procedure shall be used to determine quantitatively the amount of solute extracted in the extraction solvent.

(v) **Analysis of reference compounds.** Prior to analyzing the test solution, make duplicate runs on at least two of the reference compounds listed in table 1 in paragraph (b)(4)(ii) of this guideline. When using the reference compounds, follow the same procedure previously described for preparing the test solution and running the test. If the average value obtained for each compound is within 0.1 log unit of the reference value, then the test procedure and HPLC system are functioning properly; if not a thorough checking over of the HPLC and careful adherence to the test procedures should be done to correct the discrepancy.

(vi) **Modification of procedures for potential problems—Decomposition of the test compound.** If the test compound decomposes in one or more of the aqueous solvents required during the period of the test at a rate such that an accurate value for water solubility cannot be obtained, then it will be necessary to carry out detailed transformation studies; e.g., hydrolysis under OPPTS 830.2110. If decomposition is due to aqueous photolysis, then it will be necessary to carry out the studies in the dark, under red or yellow lights, or by any other suitable method to eliminate this transformation process.

(d) **Data and reporting—**(1) **Test report.** (i) For the test solution, report the weights to the nearest 0.1 mg of the test substance and octanol. Also report the weight percent and molar concentration of the test substance in the octanol; the density of octanol at 25 °C is 0.827 gm/mL.

(ii) For each run provide the molar concentration of the test substance in water for each of three determinations, the mean value, and the standard deviation.

(iii) For each of the three determinations calculate the octanol/water partition coefficient as the ratio of the molar concentration of the test substance in octanol to the molar concentration in water. Also calculate and

report the mean K_{ow} and its standard deviation. Values of K_{ow} shall be reported as their logarithms ($\log_{10}K_{ow}$).

(iv) Report the temperature (± 0.05 °C) at which the generator column was controlled during the test.

(v) For each reference compound report the individual values of $\log_{10}K_{ow}$ and the average of the two runs.

(vi) For compounds that decompose at a rate such that a precise value for the solubility cannot be obtained, provide a statement to that effect.

(2) **Specific analytical, calibration and recovery procedures.** (i) For the HPLC method describe and/or report:

(A) The method used to determine the sample loop volume and the average and standard deviation of that volume.

(B) The average and standard deviation of the response factor.

(C) The extraction solvent and the extraction efficiency used.

(D) Any changes made or problems encountered in the test procedures.

(ii) For the GC method report:

(A) The column and GC operating conditions of temperature and flow rate.

(B) The average and standard deviation of the average area per microliter obtained for each of the standard solutions.

(C) The form of the regression equation obtained in the calibration procedure.

(D) The extracting solvent and extraction efficiency used.

(E) The average and standard deviation of solute concentration in each collection vessel.

(F) Any changes made or problems encountered in the test procedure.

(iii) If another approved analytical method is used to determine the concentration of the test chemical in water, then all the important test conditions shall be reported.

(iv) If the concentration of the test substance in octanol is determined by an independent analytical method such as GC, provide a complete description of the method.

(e) **References.** The following references should be consulted for additional background material on this test guideline.

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